

The effect of Carbon Tetrachloride (CCl₄) and Ethanol (C₂H₅OH) on the Determination of Levels Glutathione Peroxidase, Catalase, Glucose-6-Phosphate Dehidrogenase and Lipid Peroxidation Liver and Kidney in the Goose

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Summary

The elevation of plasma and tissue lipid peroxide levels is an indicator of membrane disruption in various tissue and organ cells and it is positively correlated with the gravity of the disease.

Changes in the liver of malondialdehyde (MDA, a degredative product of lipid peroxidation) and in the antioxidant defence system in geese with alcohol and carbon tetrachloride-induced liver and kidney degeneration were studied. The experiments were carried out on 3 week-old clinically healthy (40 animals), geese (*Anser anser*) weighing 200-250 g divided into control and experimental groups of 10 animals each. The first group was used as the control group. The second group was given 1ml/kg b.w. CCl₄ while the third group was given 1.5 ml/kg b.w. CCl₄. The fourth group was given 50 % ethanol (5 ml/kg b.w). The treatment was made tree times a week for 12 weeks. 1 and 1.5 (ml/kg b.w) doses of CCl₄ and ethanol increased the malondialdehyde level of the liver as a consequence of the lipid peroxidation. At the results of this study malondialdehyde (MDA) levels was increase and glutathione peroxidase (GSH-Px), catalase (CAT), glucose-6 phosphate dehidrogenase (G6PD) were decrease as according to the liver and kidney tissue's degeneration.

Keywords: Alcohol, carbon tetrachloride, antioxidant enzyme, lipid peroxidation

Karbon Tetraklorür (CCl₄) ve Etil Alkol (C₂H₅OH)'ün Kaz Karaciğer ve Böbreklerinde Glutasyon Peroksidaz, Katalaz, Glukoz-6-Fosfat Dehidrogenaz Enzim Aktiviteleri ve Lipid Peroksidaz Düzeylerine Etkilerinin Belirlenmesi

Özet

Plazma ve dokularda lipid peroksid düzeyleri çeşitli organ ve hücrelerdeki bozunmaya bağlı hastalıklar ile pozitif bir korelasyon gösterir. Çalışmada Kaz karaciğeri ve böbreklerinde karbon tetraklorür ve etil alkol ile oluşturulan doku hasarlarında lipid peroksidasyonunun bir ürünü olan malondialdehit ve antioksidan defans sistemi araştırıldı.

Çalışmada 40 adet 200-250 g klinik olarak sağlıklı 3 haftalık *Anser anser* ırkı kaz palazı kullanıldı. Hayvanlar 4 eşit Gruba ayrıldı. Birinci grup kontrol grubunu oluşturmuştur. Bu Grup standart yem ve su ile *ad libitum* olarak beslendi. İkinci Gruba canlı ağırlık başına 1 ml/kg CCl₄, III. Gruba 1.5 ml/kg CCl₄ ve IV. gruba da %50'lik etil alkolden kilogram başına 5 ml/kg haftada üç kez olmak üzere 12 hafta boyunca oral yolla verildi.

Bu çalışma sonunda karaciğer ve böbreklerde hasara bağlı olarak malondialdehide (MDA) düzeylerinde artış, glutasyon peroksidaz (GSH-Px), katalaz (CAT), glukoz-6fosfat dehidrogenaz (G6PD) aktivitelerinde azalma görüldü.

Anahtar sözcükler: Karbon tetraklorür, etil alkol, antioksidan enzimler, kaz.

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INTRODUCTION

Experimental evidence indicates that a number of toxic and carcinogenic processes, induced by physical and chemical agents in the liver and other organs involve the formation of reactive radical species¹⁻⁵. Numerous studies have demonstrated that certain types of chemical-induced liver injury such as carbon tetrachloride toxicity may involve the peroxidation of cellular lipids^{5,6}.

Free radical-mediated peroxidation phenomena play an important role in the mechanism of cellular damage caused by both carbon tetrachloride (CCl₄) and ethanol⁶⁻⁹. Several studies on the effect of ethanol damage were conducted on organs such as the liver, stomach, central nervous system, heart, and testes, which are thought to be sensitive to ethanol intake^{10,11}. In these studies, the changes in antioxidant levels in addition to reactive oxygen species (ROS) formation were investigated following ethanol intake, and proteins, is the critical process underlying CCl₄ hepatotoxic¹². The liver is the primary site of alcohol metabolism. As alcohol is broken down in the liver, a number of potentially dangerous by products are generated, such as acetaldehyde and highly reactive molecules called free radicals⁶. Perhaps more so than alcohol itself, these products contribute to alcohol-induced liver damage¹³. Since little information is available on the effect of ethanol and CCl₄ on kidney.

An essential consequence of the development of the characteristic tissue lipid profiles will be the requirement for an effective antioxidant defence mechanism to protect the highly polyunsaturated components against peroxidative damage¹⁴⁻¹⁶. GSH and G6PD are among the major antioxidant defence systems that eliminate lipid peroxides and toxic oxygen radicals. G6PD (d-glucose 6-phosphate: NADP + oxidoreductase, EC 1.1.1.49) is a key enzyme which catalyses the first step of the pentose phosphate metabolic pathway¹⁷. A major role of NADPH in erythrocytes is regeneration of reduced glutathione, which prevents haemoglobin denaturation, preserves the integrity of red blood cell membrane sulphhydryl groups, and detoxifies hydrogen peroxide and oxygen radicals in and on the red blood cells¹⁸. Catalase is an important intracellular enzyme of the antioxidant system. By decomposing the hydrogen peroxide (H₂O₂) to water and oxygen, it protects the cells from oxygen-free radicals which are formed in the presence of high amounts of H₂O₂¹⁹. The modulation of catalase activity is one of the cellular defence mechanisms by which organisms try to lessen the potential toxicity of H₂O₂^{20,21}.

In addition, GSHPX activity removes hydrogen peroxide *in vivo* using GSH and helps to prevent hydroxyl radical formation²². Furthermore, its activity may be used to assess body selenium status and nutritional requirements.

In this study the pathological effects of different doses of CCl₄ and ethyl alcohol on MDA levels, and GSH-Px, CAT, G6PD activities in the livers and kidney of geese were examined and compared.

MATERIAL and METHODS

Animals and Treatment: The experiments were carried out on 3 week-old clinically healthy (40 animals), geese (*Anser anser*) weighing 200-250 g divided into control and experimental groups of 10 animals each. The animals were fed with standard pellet diet. The diet provides 2910 kcal kg⁻¹²³. They were kept in a room with controlled temperature (23 ± 2°C) relative humidity (60 ± 10 %). An automatic timer controlled the light on a 12 h light/12h dark cycle.

The first group was used as a control group. The second group was given CCl₄ (1ml/kg b.w.); the third group was given with 1.5 (ml/kg b.w.) CCl₄ and the fourth group was given ethanol 1:1 w/w (5ml/kg b.w.) three times a week for 12 weeks.

Biochemical Analysis of Tissues: Geese were sacrificed, under ether anaesthesia after 12 weeks. Liver and kidney were immediately excised. Liver and kidney stored at -70°C for subsequent assays. The tissues were weighed, rinsed with ice-cold deionised water, cut into small pieces, then dried with filter paper and homogenized using the appropriate buffer depending upon the variable to be measured. The homogenates were centrifuged at 700 g for 10 min and recentrifuged at 16,500 g for 20 min at 4°C to obtain a post nuclear homogenate and post mitochondrial supernatant fractions.

In the liver and kidney, lipid peroxidation in tissues was measured by the thiobarbituric acid reacting substance (TBARS) method of Placer et al.,²⁴ and was expressed in terms of the malondialdehyde (MDA) content, which served as standard of 1.1.3.3-tetraethoxypropane. Values were expressed as MDA equivalents in nmol/g tissue. Glutathione peroxidase, glucose-6-phosphate dehydrogenase activities were measured according to Beutler's (Beutler et al., 1975) method²⁵. Catalase levels were determined using the methods described by Aebi²⁶. The catalase mediated

the decomposition of H₂O₂ was followed directly at 240 nm. Protein concentration was according to Lowry's 27.

Statistical Analysis: Group II, III and IV were compared with controls (Group I). The collected values as mean± SE were statistically analyzed (student's t-test) (IFFC, 1987) and evaluated by spss 6.0 (1993) packed program p value<0.01, 0.05 were considered significant²⁸.

RESULTS

Table I shows that debating intake of CCl₄ and ethyl alcohol induces significant reduction of the liver and kidney levels.

The liver and kidney of CCl₄-dosed (1ml/kg) geese contained 16.2±11, 8.4±0.22 μmol/g MDA, 46.3 ±1.3, 52±22 U/g protein GSH-Px, 52 ±22, 146.4 ±6.7 k/g proteins CAT and 32±13, 14.8±1.2 U/g protein G6PD (table 1). The activities of GSH-Px, and CAT enzymes, expressed/g prot, were considerably higher in the 1.5 CCl₄-dosed and ethyl alcohol geese.

Experimental of the groups G6PD activity was decreased (p<0.01). Significant increases of liver MDA (p<0.01) occurred with CCl₄ and ethyl alcohol-dosing.

Table 1. The MDA levels and GSH-Px, CAT, G6PD activity in control and CCl₄, C₂H₅OH- induced treated geese.

Table 1. CCl₄ ve C₂H₅OH ile muamele edilen kazlarda ve kontrol gruplarında GSH-Px, CAT, G6PD ve MDA düzeyleri.

	MDA (μmol/g)	GSH-Px (U/g protein)	CAT (k/g protein)	G6PDH (U/g protein)
Control (n:10)				
Liver	8.9±16	51±1.2	186±22	47.3±14
Kidney	6.3±0.17	58±2.8	176.2±10.4	25±0.2
Treatment (n:10) (1ml/kg CCl₄)				
Liver	16.2±11**	46.3±1.3**	152.2±9.7**	32±13**
Kidney	8.4±0.22**	52±22**	146.4±6.7**	14.8±1.2**
Treatment (n:10) (1.5ml/kg CCl₄)				
Liver	27±23**	46.6±3.2**	170±10.8**	38.7±22**
Kidney	9.1±2.2**	51.±14**	155±0.7**	9.8±16**
Treatment Ethanol (n:10) (50%, ml/kg)				
Liver	15.3±2.2**	48.3±13**	178±17*	32±16**
Kidney	7.8±13**	44.5±2.2**	158.2±0.8**	18±1.7**

p<0.01** p<0.05*

DISCUSSION

Animals can not drink large amounts of ethanol and CCl₄ spontaneously, but can be easily intoxicated by forced ethanol administration. It is possible to study the effects of chronic alcohol and CCl₄ consumption, on different organs and tissues, by wing geese which have been bred selectively for high ethanol preference.

Much of the direct cell damage that occurs during liver disease is believed to be caused by free radicals. Free radicals are highly reactive molecular fragment that frequently contain oxygen²⁹. Small quantities of free radicals are produced as normal by-products of various metabolic processes. These fragments are quickly scavenged by natural protective molecules in the cell, called antioxidants (GSH, GSH-Px, SOD, vitamin A and E). However, when free radicals are produced in excess or when antioxidant defences are impaired, the free radicals may in treats destructively with vital cell constituents, potentially causing death of the cell^{3,6}.

Ethanol is a widely consumed sedative-hypnotic drug throughout the world³⁰. It has been shown that ethanol intake may lead to oxidative damage in several tissues such as brain, stomach, liver or erythrocyte^{31,32}.

Since ethanol has oxidant effects in erythrocytes and cell, it was considered important to reveal the effect of ethanol consumption on erythrocyte G6PD activity, which has not been studied before. Therefore the objective of this study was to investigate the effect of ethanol on GSH-Px, G6PD, CAT enzyme activity in geese liver and kidneys.

In fact, ethanol causes several haemolytic disorders due to both direct and indirect effects³⁰.

We examined the individual effects of ethanol and CCl₄ on liver and kidney. We found that treatment of geese with ethanol and CCl₄ for 12 weeks significantly reduced GSH-Px, CAT, G6PD content and elevated the MDA level in the liver and kidney.

Free radicals are involved at the early stages of CCl₄ intoxication³³. The CCl₄ and ethanol that we used in this study are frequently used to damage liver and their peroxidant activity is known²⁴. It is well known CCl₄ injures hepatic cells through free-radical-induced lipid peroxidation and direct free radical attract³⁵⁻³⁷. In our study, maximal inhibition of G6PD, GSH-Px, and CAT activity

were within 12 weeks after 1.5 ml CCl₄ administration.

The prevailing explanation of CCl₄ toxicity is that the toxicity of CCl₄ is based on lipid peroxidation caused by the trichloromethyl radical. In the present study, we found that CCl₄ treatment markedly increased the MDA formation.

MDA levels increased all group. These results show that (1 ml/kg) after the treatment of animals with low and moderate (1.5 ml/kg) dose of CCl₄ the LPO level is found to be considerably high. Similar results were reported by 7 who used the same technique as we did in this study.

Hepatic GSH-Px, CAT, G6PDH and, SOD may play an important role in the detoxication of xenobiotics, including CCl₄²⁶ in fact, there are reports that the increase in CCl₄-induced hepatic injury by starvation results from the decrease in antioxidant enzyme content^{7,33,35}.

The latter point is important since oxidative stress depletes intracellular glutathione which, thereafter, is restored by the enzyme GSH which utilise reductive equivalents furnished by G6PD, a major component of the pentose shunt pathway. However, further studies are necessary to support these results. For example, the significance of this enzyme in liver and kidney tissues may be investigated by repeating the experiments in different time periods using different doses.

In conclusion, in Anser anser geese with induced liver degeneration, the levels in liver and kidney tissue of the antioxidant defence system constituents GSH-Px, CAT, G6PD and lipid peroxidation are reported here in detail for the first time.

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